HEMOLYTIC STREPTOCOCCI IN THE THROAT IN CERTAIN ACUTE INFECTIOUS DISEASES

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To determine the prevalence of hemolytic streptococci in the throat of patients with acute infectious diseases some 300 patients have been examined.

Swabs were taken from the mouth and nose, as well as from the throat, of each patient. In swabbing the throat the tonsils and pharynx were always touched. The mouth included the cheeks, gums and the floor, also of course bringing along more or less saliva. In the nose attempts were made to reach the nasopharynx, but these were not always successful, many of the patients being very young children. After the swabs were taken, the methods adopted by the Medical Department of the United States Army for the isolation and identification of hemolytic streptococci[1] were followed with the exception that goat's blood was used for blood agar instead of horse, rabbit or human blood. The surface inoculation of blood agar plates was used and the organisms that answered to the current descriptions of hemolytic streptococci on blood agar plates, formed chains in broth, did not dissolve in bile and laked a 5% suspension of rabbit corpuscles in 2 hours, were considered as such.

The organisms in question, as usually described, formed small, grayish, shiny, moist, elevated, round colonies, surrounded by a clear zone of hemolysis about 2-4 mm. in diameter. Most of the strains of streptococci obtained in my work answered to this description, but all the strains were not exactly alike on blood agar plates. In general, strains obtained from healthy persons seemed like those obtained from patients, and no difference was noticed in strains from different sources in the same individual. On surface plates the colonies of some strains were very small in size, dense, opaque and of a grayish white color, surrounded by a comparatively large, completely cleared zone. The colonies of other strains were as large as 8 mm. in diameter, flat, slightly elevated, of a light grayish color, dull and finely granular. Other strains showed a rather small hemolytic zone compared to the size of the colony. All strains produced complete hemolysis, yet there was a difference in the clearness of the laked area, although the microscope revealed that all cells within the hemolytic zone had been laked. The colonies of most strains were round and convexly elevated, but some were lobulated or irregularly outlined; others were larger in size, flat, elevated; while still others showed a rounded raised edge with a slight central flattened depression. Most of the deep colonies were biconvex, but lobulated or irregularly outlined and triangular colonies were also seen. The color of the colonies varied from brownish to whitish.

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[1] Recommendations of the Committee on a Standard Routine Method for the Isolation and Identification of Hemolytic Streptococci from Throats, Sputa, and Pathologic Exudates. Jour. Lab. and Clin. Med., 1918, 3, p. 618; Jour. Med. Research, 1915, 31, p. 455.

The differences noted in the appearance of the colonies of different strains could not well be due to the blood agar as this was always fresh and plates of uniform thickness were used.

In the corpuscle suspension a few strains caused complete hemolysis in one hour. There was a tendency in certain strains of the stock culture to lose some of the hemolytic properties, this being more marked in the 5% suspension of rabbit corpuscles than on blood-agar plates. In most strains no difference was noticed. Fermentation tests of 50 strains gave results (table 1) showing that, according to Holman's classification,[2] the strains fall into the pyogenes and anginosus groups.

TABLE 1

SUGAR REACTION OF FIFTY STRAINS

Sugar	Lactose		Saccharose		Raffinose		Mannite		Inulin		Salicin	
	+	−	+	−	+	−	+	−	+	−	+	−
Number of strains....	50	0	50	0	0	50	4	46	0	50	2	48

In 140 of the 300 patients, or 46.6%, examined by the methods outlined, hemolytic streptococci were present at the time of entrance into the hospital; 40 more became positive during their stay in the hospital, making a total of 180 or 60% giving positive cultures. Of the 180 positive cases, 96 or 53.3% became negative while in the hospital, 84 or 46.6% remained positive. In 160 patients, or 53.3%, the cultures gave a negative result and 120, or 75%, of these remained negative while 40, or 25%, became positive while in the hospital. Of the 300 patients, 130 had diphtheria, of whom 58 or 46.6% gave positive cultures, and 125 had scarlet fever, of whom 98 or 78.4% gave hemolytic streptococci. The remaining 45 had miscellaneous diseases with 20 or 44.4% positive cultures. From a very small number of the patients would be obtained a positive culture from all three sources—mouth, throat and nose. Positive cultures were obtained from the mouth and throat of a larger number of patients, and from the throat and nose of a still larger number, while positive cultures were taken from the throat of all patients with positive cultures. Patients from the nose of whom positive cultures were obtained did not as a rule have streptococci in the mouth, and vice versa. In all, 50 positive cultures, or 16.3%, were obtained from the nose, and 33 positive cultures, or 11%, from the mouth. In the scarlet fever patients 20.8% positive cultures were obtained from the nose, and 12.8% from the mouth. Of the diphtheria patients 10% positive cultures were obtained from the nose and 4.6% from the mouth. The percentage of positive cultures in the miscellaneous diseases was alike for mouth and nose, being 11% from both sources. Apparently, the throat is the area of predilection while the nose, and especially the mouth, do not seem to harbor the organisms frequently. When hemolytic streptococci were present in the nose, they were very often the predominating organisms there; this was not true in the mouth where, as a rule, they were found in small numbers. The nasal secretions apparently furnish a good medium for the development of the streptococcus. In the throat the plates would give from a few colonies to an almost pure growth, but the streptococcus was never found in pure culture in any of the three sources. The great majority of these patients were children, most of whom had more or

[2] Jour. Med. Research, 1916, 34, p. 377.

less enlarged tonsils. Swabs were also taken, in the manner described, from 33 nurses on duty and 7 of these, or 21.2%, proved to be carriers of hemolytic streptococci—a rather low percentage, due perhaps to the protection afforded by the wearing of masks. Of a class of average healthy medical students, 57% gave positive cultures of streptococci.

It was noticed that a large number of the negative cases which later became positive did so after being placed with a carrier or carriers of the organism in question. This was not always true as some patients persisted in giving negative cultures although they were in a room with carriers. When several members of one family are ill, it is the rule to place these patients together as much as circumstances permit, and in my experience all became carriers of hemolytic streptococci. In only a few instances was this not true. The best example is that of a family of six in a ward: One patient gave a positive culture from the beginning, while in the remaining five the organisms could not be demonstrated at any time during the illness Patients giving a positive culture at the time of their entrance into the hospital and later becoming negative would usually become so gradually, although the decrease in the number of streptococci was not parallel with the recovery of the patient. Nor did the number of organisms in patients that did not become negative always seem to be influenced by recovery. Many patients showed as large a number of organisms in their throats when well and ready to be discharged as they did at any time during their sickness.

The virulence for animals of the organisms I studied was low: 1 c c of a fresh broth culture did not seem to affect rabbits and 0.5 c c did not always kill a mouse. If phagocytability can be used as a guide to virulence, the hemolytic streptococci in question were not very virulent. Nearly all the strains tested were taken up by the human leukocytes in the presence of normal human serum in large numbers. In order to test the organisms as soon as possible after their removal from the body, swabs were inoculated into broth, incubated for from 4-6 hours, then phagocytosis experiments made. No chains of cocci were seen within the leukocytes. Diplococci and masses of individual cocci were seen, but it was not possible to say whether these cocci were hemolytic streptococci or pneumococci and staphylococci. As soon as pure cultures of hemolytic streptococci could be secured marked phagocytosis resulted. Strains obtained from other sources were not so uniform in this respect. Two strains from suppurating glands of the neck in scarlet fever patients did not give any phagocytosis at the end of 4 hours' cultivation in broth, but they did after the first subtransfer. Another strain which had been passed through mice was not taken up by leukocytes after being transferred directly from the mouse to serum broth, but it was taken up on the first subtransfer from the serum broth to a blood-agar slant. Several strains from cases of erysipelas were phagocytable as was one strain from a severe case of streptococcic septicemia. Several strains from pleural exudates from influenzae-pneumonia patients, one strain from a peritonsillar abscess, and one from the suppurating ear of a scarlet fever patient gave no phagocytosis when tested in the manner indicated, but they all soon became phagocytable on artificial growth. One strain from the spinal fluid of a case of streptococcic meningitis was nonphagocytable. This strain was not tested after growth on artificial medium. One strain obtained from the blood of a scarlet fever patient shortly before death of the patient was nonphagocytable after the eleventh subtransfer. Whether or not phagocytosis took place, there

seemed to be no destruction or change of the leukocytes that came in contact with the streptococci. In pus and other exudates examined phagocytosis (by the patient's pus cells) was noted in many instances, so also in the leukocytes of mice that had been killed by the streptococcus. In the exudates there was also a destruction of leukocytes, but whether or not this was caused by the organisms present, could not be said. Pus cells are always more or less degenerated even in the absence of organisms. The report of the empyema commission of the U. S. Army states that in smears of fluid obtained by aspiration of the pleural cavity the majority of leukocytes present had undergone degeneration; also that in many cases there was no evidence of phagocytosis. In other, but fewer cases, phagocytosis was very marked. It seems that the resistance of hemolytic streptococci to opsonins may be lost rapidly on artificial cultivation; also that in some individuals opsonins are either absent or not present in a large enough amount to produce phagocytosis.

COMPARISON OF RESULTS OBTAINED BY DIFFERENT METHODS

According to the committee on standard methods,[1] if streptococci are being sought for in material in which they may be quite rare, a preliminary growth in serum broth, glucose blood broth or cooked meat medium will serve to encourage this more than that of other organisms. If, on the other hand, it is desired to know the relative numbers of streptococci and other organisms present in the original material, it should be plated directly without preliminary enrichment. The recommendations also state that for routine examination of swabs in large numbers surface inoculation only of blood agar plates is sufficient. As stated, the latter method was followed in my work, and many of the recent investigators have also followed this method. As will be seen from tables 2 and 3, the percentage of streptococci obtained from various sources by different investigators, varies considerably. Some observers have found hemolytic streptococci in a much lower percentage from the same source. One reason for this is that different observers used different methods. Some followed the enrichment method, others washed the swab in a tube of melted blood agar and poured this on plates, while still others used the surface inoculation method.

After most of my observations had been made, the work was repeated on a small number of cases, using both the surface inoculation method and the enrichment method, in order to determine which method gave the highest number of positive cultures. Of 20 cases of scarlet fever, 19 or 95% gave positive cultures by the enrichment method, while 16 or 80% did so when the surface plate method was followed. Of 9 healthy individuals, one showed hemolytic streptococci by the latter method and 3 by the enrichment method. The class of medical students mentioned gave 46% by the surface plate method. According to these results, surface inoculation only of blood-agar plates as recommended is not sufficient for accurate work. A very small number of streptococci may have been picked up by the swab and in the process of inoculation of the plate the area of the swab containing the streptococci may not touch the blood agar at all, or the colonies may be so few in number that a hemolytic zone of a hemolytic staphylococcus, diphtheria bacillus or some other hemolytic organism may enclose and therefore obscure the small streptococcus colony that might be present. Also, the better growing organisms may entirely outgrow and cover the streptococci making it impossible to determine

[1] The Empyema Commission: Cases of Empyema at Camp Lee, Va., Jour. Am. Med. Assn., 1918, 71, p. 443.

their presence. We do not, as a rule, know whether the streptococci sought for are rare or numerous in the material to be examined, and the preliminary enrichment method certainly seems to yield more accurate results than the surface inoculation. The swab should be incubated in a liquid medium for a few hours, then inoculated into a tube of melted blood agar and poured in a plate. In this way the streptococci present will have become more numerous, and as the other organisms present do not grow as rapidly as on a surface plate, they will not interfere with the growth of hemolytic streptococci to such an extent that the latter are crowded over.

TABLE 2

INCIDENCE OF HEMOLYTIC STREPTOCOCCI FROM VARIOUS SOURCES IN VARIOUS DISEASES

Disease	Mouth		Throat		Nose		Sputum		Investigators
	No. of Cases	% Positive	No. of Cases	% Positive	No. of Cases	% Positive	No. of Cases	% Positive	
Influenza and pneumonia	....	....	....	....	....	4	....	20	Nuzum and others[4]
Measles	....	....	388	77.1	....	....	....	....	Levy and Alexander[5]
Influenzal bronchopneumonia	....	....	....	4	....	....	....	....	Keegan[6]
Influenza	....	....	....	4	....	....	....	....	Friedlander and others[7]
Bronchopneumonia	....	....	159	12.2	....	....	....	....	Hirsch and McKinney[8]
Tonsillitis	9	33.3	9	88.2	9	66.6	....	....	Nichols and Bryan[9]
Influenzal pneumonia	....	....	79	5	....	....	302	0	Hall, Stone and Simpson[10]
Influenzal pneumonia	....	....	366	34	....	....	740	17	Blanton and Irons[11]
Influenza	....	....	....	....	....	....	20	65	Davis[12]
Influenza	....	....	....	....	....	....	42	31	Davis[12]
Arthritis, nephritis, myocarditis	....	....	48	94	....	....	....	....	Davis[12]
Influenzal pneumonia	....	....	....	....	....	....	69	4.3	Opie, Freeman et al.[13]
Bronchopneumonia	....	....	....	....	....	....	43	14	Opie, Freeman et al.[13]
Measles	....	....	857	35	....	....	....	....	Cumming and others[14]
Lobar pneumonia	....	....	165	69	....	....	92	41	Cumming and others
Scarlet fever	....	....	192	87	....	....	....	....	Cumming and others
Otitis media	....	....	204	74	....	....	....	....	Cumming and others
Mumps	....	....	147	37	....	....	....	....	Cumming and others
Tonsillitis	....	....	79	54	....	....	....	....	Cumming and others
Pneumonia	....	....	....	....	....	....	156	30	Lamb[15]
Pneumonia and measles	....	....	....	5.7 to 20	....	....	....	....	Beals and others[16]
Pneumonia empyema	....	....	....	33 to 40	....	....	....	....	Beals and others
Acute infectious disease	300	11	300	60	300	16.3	....	....	Otteraaen
Diphtheria	130	4 6	130	46.6	130	10	....	....	Included in 300 cases of acute infectious diseases
Scarlet fever	125	12.8	145	82	125	20.8	....	....	

[4] Ibid., p. 1562.
[5] Ibid., 1918 70, p. 1827.
[6] Ibid., 1918, 71, p. 1051.
[7] Ibid., p. 1652.
[8] Ibid., p. 1735.
[9] Ibid., 1918, 71, p. 1813.
[10] Ibid., p. 1986.
[11] Ibid., p. 1988.
[12] Ibid., 1919, 72, p. 819.
[13] Ibid., 1919, 72, p. 108 and p. 556.
[14] Ibid., p. 704.
[15] Ibid., p. 1133.
[16] Jour. Infect. Dis., 1918, 23, p. 475.

TABLE 3

INCIDENCE OF HEMOLYTIC STREPTOCOCCI FROM VARIOUS SOURCES IN HEALTHY PERSONS AND FROM EXCISED TONSILS

Throat		Nose		Excised Tonsils		Investigators
Number of Cases	Percentage Positive	Number of Cases	Percentage Positive	Number of Cases	Percentage Positive	
489	14.8	....	....	....	....	Levy and Alexander[5]
95	83.2	....	....	....	....	Levy and Alexander
50	28	....	....	100 pairs	75	Nichols and Bryan[9]
		357	75	....	....	Blanton and Irons[11]
284	26	....	....	....	....	Opie, Freeman and others[13]
4,743	1	....	....	....	....	Hamilton and Havens[17]
42	60	....	....	61 pairs	97	Davis[12]
168	6	69	55	142	82	Cumming, Spruit and Aten[14]
308	1.6	....	....	....	....	Bunce, Berlin and Lawrence[18]
35	57	....	....	....	....	Otteraaen
33	21.2	....	....	....	....	Otteraaen

CONCLUSIONS

Hemolytic streptococci are frequent inhabitants of the throats of normal individuals and of persons suffering from acute infectious diseases. These streptococci are not virulent so far as indicated by the results of animal inoculations and phagocytoses experiments. The enrichment method should be used in preference to the surface plate method in examinations for streptococci.

[17] Jour. Am. Med. Assn., 1972, p. 272.
[18] Ibid., p. 782.